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SOP14v1_TGD_DNAExtraction(QiagenDNeasyBlood&TissueKit)

DOI

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Protocol status: Working

We use this protocol and it's working

Created: August 12, 2020

Last Modified: October 01, 2024

Protocol Integer ID: 40360

Keywords: biohazardous waste container, liquid waste, solid waste container, liquid waste into the appropriate bucket, waste disposal, reagent hazards ethanol, liquid waste bucket, dna bench, waste disposal all flow, other plastic, reagent bottle, plastic, dna, blue dna, tissue, labeled material, reagent, contact with tissue, ethanol, lab attire

Abstract

Guidelines

Bench work should be completed on the **pre-PCR/DNA bench**.

Blue DNA-only labeled materials should be used for this protocol to avoid cross-contamination.

Lab attire: gloves, lab coat, and safety glasses/goggles.

Before starting

Add Proof 200 ethanol to Buffer AW1 and Buffer AW2 as indicated on the reagent bottle.

Preheat dry bath to 56 C.

Reagent Hazards

Ethanol - highly flammable, skin and eye irritant, acutely toxic.

Waste Disposal

All flow-through/liquid waste goes in liquid waste bucket and can be disposed of in the trash.

Plastics that come into contact with tissue should be collected and disposed of in the biohazardous waste container.

All other plastics (columns, tips, tubes) go in solid waste container (after pouring the liquid waste into the appropriate bucket) and can be disposed of in the trash.

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Materials

MATERIALS

 DNeasy Blood & Tissue Kit, QIAGEN **Catalog #Cat No./ID: 69504**

Ethanol/Ethyl Alcohol, Proof 200 - Gold Shield Distributors - Catalog Number 412811

Safety warnings

Reagent Hazards

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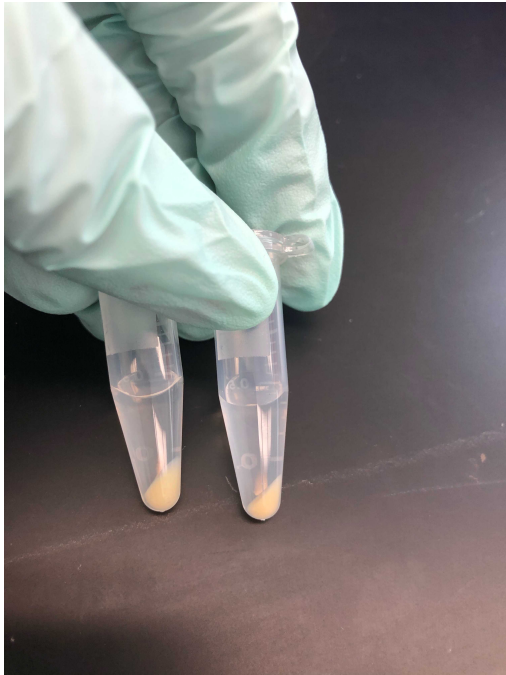
Before start

Add Proof 200 ethanol to Buffer AW1 and Buffer AW2 as indicated on the reagent bottle.

Preheat dry bath to 56°C.

- 1 Take a portion of only cell pellet that is less than about 30 uL in size to ensure efficient lysing and flow-through, and put into a new microcentrifuge tube.

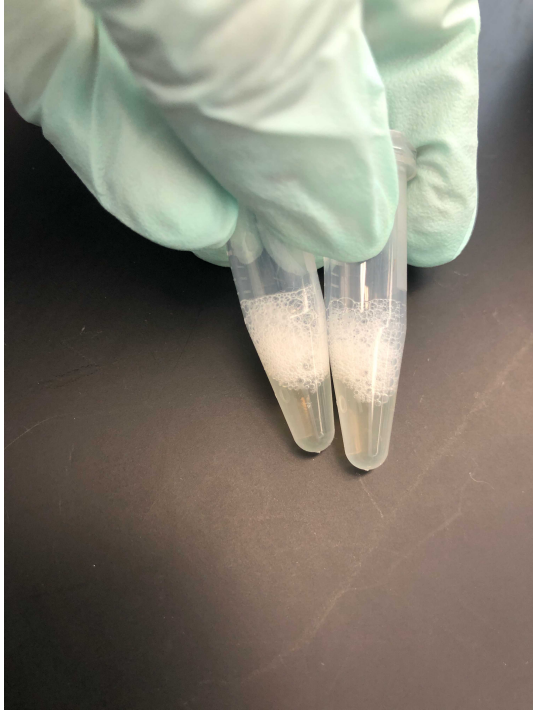
1.1



For reference, this pellet is about 50 uL in size.

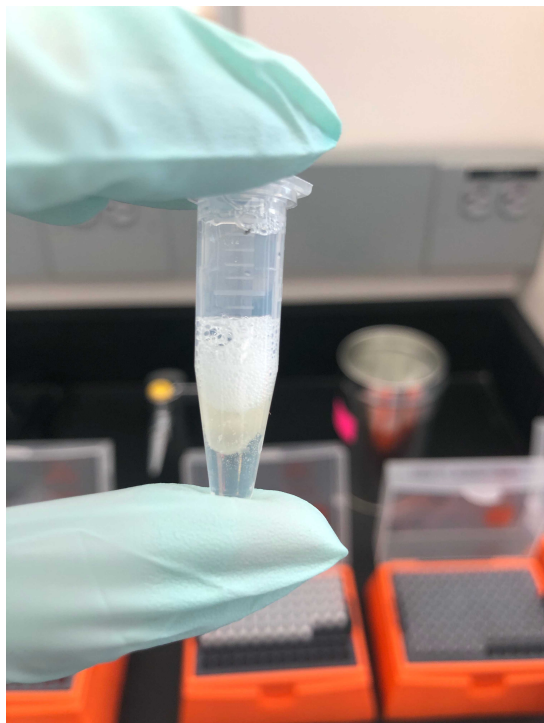
- 1.2 If cell pellet is submerged in buffer solution (such as PBS), you need to mix the cell pellet and the PBS by pipetting and then take a portion of it that you estimate will contain 30 uL of cell pellet. Spin down the portion (8000xg for 5 minutes at room temperature) and remove the supernatant. If only cell pellet is provided i.e. it is not submerged in PBS or any other solution, note down volume of cell pellet present, add in 1 mL of PBS, mix, take enough of the mixed solution to get a 30 uL pellet, spin down at 8000xg for 5 minutes at room temperature, and remove the supernatant to get an approximately 30 uL pellet.
- 2 Resuspend the pellet in 180 uL of Buffer ATL (lysis buffer). Add 20 uL proteinase K. Vortex to mix.
- 3 **OPTIONAL:** Add 4 uL of 100 mg/mL RNase A and vortex to mix. Incubate for 2 min at RT.
**If RNA-free genomic DNA is required
- 3.1 **REMINDER:** RNase A should never come in contact with **RNA bench** or any **pink RNA-only** materials.

- 4 Incubate at 56°C for 20 minutes.
- 4.1 Make sure pellet is completely lysed. Vortex occasionally and mix with a p1000 pipet after the first 20-minute incubation.



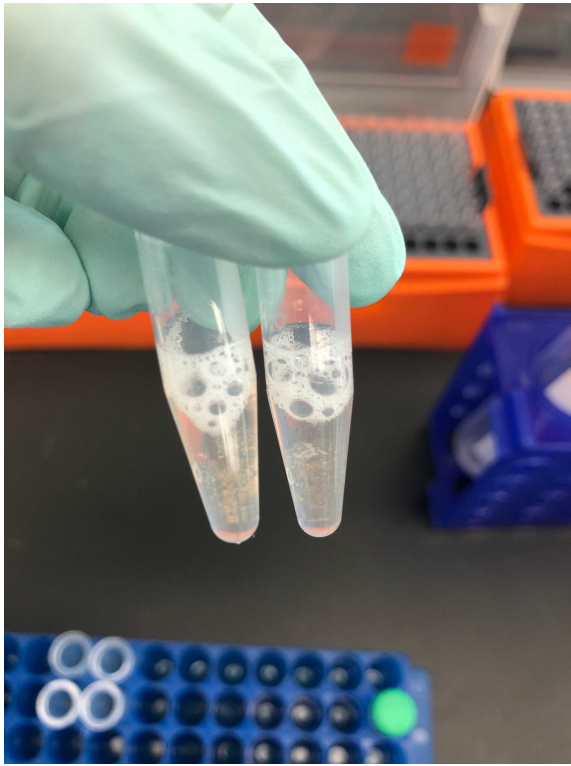
- 5 Add 200 uL of AL Buffer. Vortex to mix and then use a p1000 pipet to homogenize the solution as much as possible. Incubate at 56°C for another 20 minutes.

5.1



- 6 Then, add 200 μ L of 96-100% ethanol to sample and vortex to mix. Use a p1000 pipet first then a p200 pipet to homogenize the solution. Solution should be homogenous.

6.1



- 7 The following steps involve spinning down columns and collecting flow-through. If you have a lot of cellular lysis debris that inhibits flow-through, you can spin down the tube a **second** time for each of the steps, at a higher speed.
- 8 Transfer mixture to DNeasy Mini spin column, placed in 2 mL collection tube. Centrifuge at 9000 x g for 1 minute (RT). Discard flow-through in liquid waste bucket, and collection tube in solid waste container.
- 9 Place column into new 2 mL collection tube. Add 500 uL of Buffer AW1. Centrifuge at 9000 x g for 1 minute (RT). Discard flow-through in liquid waste bucket, and collection tube in solid waste container.
- 10 Place column into new 2 mL collection tube. Add 500 uL of Buffer AW2. Centrifuge at 20,000 x g for 3 minutes (RT) to dry the column membrane. Discard flow-through in liquid waste bucket, and collection tube in solid waste container.
- 11 Place column into 1.5 mL microcentrifuge tube. Add 40 uL Buffer AE directly onto column membrane. Incubate at RT for 1 minute. Centrifuge at 9000 x g for 1 minute (RT) to elute.



- 12 **OPTIONAL:** Repeat elution in previous step to increase yield. Can use eluate from previous step (recommended), or use an additional 40 uL of Buffer AE and a new microcentrifuge tube to collect secondary eluate.
- 13 Store samples at 4°C for immediate use, -20°C for short term storage and -80°C for long-term storage.
- 14 **QUANTIFICATION:** Use Nanodrop located in Rm. 2265.
Use 1uL of Buffer AE as the blank and 1 uL of each of the samples to determine the concentration.
A260/A280 ratio of about 1.8 indicates "pure" DNA.
To check for other contamination: A260/A230 ratio of 2.0-2.2 indicates "pure" nucleic acid.